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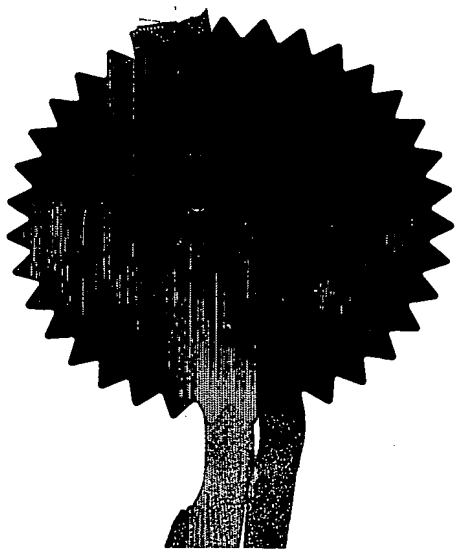

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1. Your reference	LEIE / P26861GB	
2. Patent application number *(The Patent Office will fill in this part)*	0323378.0	
3. Full name, address and postcode of the or of each applicant *(underline all surnames)*	**University of Leicester** University Road Leicester LE1 7RH United Kingdom	07OCT03 E842525-2 002866 P01/7700 0.00-0323378.0
Patents ADP number *(if you know it)*	00798348001	
If the applicant is a corporate body, give the country/state of its incorporation	United Kingdom	
4. Title of the invention	THERAPEUTIC AGENT	
5. Name of your agent *(if you have one)* "Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	**ERIC POTTER CLARKSON** **PARK VIEW HOUSE** **58 THE ROPEWALK** **NOTTINGHAM** **NG1 5DD**	
Patents ADP number *(if you know it)*	**1305010** ✓	

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a) *any applicant named in part 3 is not an inventor; or*
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11. I/We request the grant of a patent on the basis of this application.

Eric Potter Clarkson.

Signature
ERIC POTTER CLARKSON

Date
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12. Name and daytime telephone number of person to contact in the United Kingdom

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THERAPEUTIC AGENT

The present invention relates to platelet substitutes (also called synthetic, or artificial, platelets) that are useful for treating patients with deficiencies in their own platelets, such as hereditary or acquired defects of platelet numbers (thrombocytopenia) or function (thrombasthenia).

The body controls bleeding by forming blood clots. In order that a stable, insoluble, blood clot can form, and stop bleeding, a number of different components, of which the most important are thrombin, fibrinogen and platelets, need to be present at the site of a wound. Damaged tissue at the site of the wound releases tissue factor, which activates the coagulation cascade leading to the production of the enzyme thrombin. Thrombin converts fibrinogen, a soluble plasma protein, to an insoluble polymer, which is an essential part of the clot. Also present at the site of the wound are activated platelets. Platelets are the smallest cellular component of blood, and, once activated, platelets also form an essential part of a blood clot. In an initial step, platelets will adhere to the exposed wound surface and become activated. One of the platelet membrane glycoproteins, GPIIb/IIIa, undergoes a shape change, which allows it to bind fibrinogen. Fibrinogen is bipolar, which means it can bind to more than one platelet, and consequently platelets aggregate together. Platelet aggregates form the basic architecture of the clot, formed within a mesh of fibrin.

It can be seen that in the absence of any one of the three components (thrombin, fibrinogen or platelets), the fibrin clot will fail to form properly and bleeding will fail to stop. Hereditary or acquired defects of platelet numbers (thrombocytopenia) or function (thrombasthenia) can be caused as a result of decreased production of platelets by diseased bone marrow, for example in malignancy such as leukaemia or as a result of cytotoxic

therapy, or as a result of an increased rate of clearance from the circulation, for example in the case of an immune response to platelet antigens.

Platelet transfusion is currently the only effective treatment for acute bleeding and the prevention of bleeding in patients with disorders of platelet production and/or function. The 1997 Consensus Conference on Platelet Transfusion highlighted concerns about the ever-increasing demand for platelets. In the final statement of the conference it was concluded that, while there is extensive clinical evidence that platelet transfusions are valuable, the procedure carries risks and costs, raising the ethically crucial issue of balancing these with benefits.

The current standard preparation of a platelet concentrate is a suspension of platelets in autologous plasma prepared by centrifugation of whole blood (buffy coat preparations) or by apheresis. The shelf life of the concentrates is a balance between the competing needs to maintain platelet function and integrity (for which storage is optimal at 22°C), and to minimise bacterial growth (for which storage is optimal at 4°C). This conflict is resolved by storing platelet concentrates at 22°C, but restricting their shelf life to 5 days to minimise bacterial contamination. However, even over this time platelets become steadily activated. The short duration of storage and increasing clinical demand for new therapeutic regimes are resulting increasingly in shortages in supply worldwide.

Despite stringent conditions of preparation and storage, platelet transfusion is still associated with risk of acute bacterial infection. The very low but finite risk of transmitting blood-borne viruses is also well recognised, and more recently there is recognition of the theoretical risk for transmission of vCJD. This risk has been thought to be associated with leucocytes in the concentrates and may therefore be reduced, or eliminated, by

leucodepletion. However, it has been observed that platelets also carry normal prion protein, which is released during storage. Although it is yet to be established whether platelets can also carry the variant prion protein, this is of concern.

The presence of leucocytes in platelet preparations poses additional risks. It increases the risk of immunisation to HLA antigens, which can result in multi-transfused patients becoming refractory to platelets. In addition, on storage, leucocytes can release pyrogenic cytokines, adding to the possibility of an adverse reaction. For these various reasons leucocytes are now routinely depleted from platelet concentrates, but this results in a concomitant reduction in platelet yield and increased cost. In addition, leucodepletion does not remove the issue of platelet-derived cytokines (such as TGFβ and RANTES) that have also been associated with allergic reactions to platelet concentrates.

The problems associated with platelet transfusion have stimulated the search for alternatives and, to date there have been broadly three different approaches.

Attempts have been made to stabilise platelets, or fragments of platelets, to prolong their shelf life, and facilitate the application of bacterial and viral inactivation procedures. Treatment of platelet concentrates with Psoralen, a photochemical agent, and ultraviolet light has been shown to inactivate bacterial and viral pathogens in platelet concentrates. An alternative approach has been to lyophilise preparations of platelet membrane fragments, which have been shown to be transiently, and variably, effective in a limited number of patients. These approaches are still, however, limited by the fact that platelets are a highly variable supply of raw material,

which is likely to prove incompatible with reproducible manufacture and quality control.

A second approach is the use of an agent which will stimulate endogenous platelet production for example recombinant growth factors such as, thrombopoeitin or Interleukin 11. Although effective in stimulating endogenous platelet production, this approach is also limited because there is a lag between treatment and the recovery of significant platelet numbers in the blood. As a consequence such therapy is inappropriate for the treatment of an acute bleed. In addition, differences in patient response may result in either underproduction or overproduction of platelets, which can put the patient at risk of bleeding or thrombosis respectively.

The third approach is the development of non-platelet-derived haemostatic agents. The advantage of this approach is the potential to design a sterilised, lyophilised product that can be manufactured cost effectively on a large scale, using non-platelet-derived, biocompatible, specified raw materials. The aim is to develop a particulate material that has the ability to interact with residual platelets at a damaged site in a blood vessel, whilst not inducing a thrombotic reaction in the absence of vascular trauma. To do this it is important to design a product that mimics closely the action of native platelets. Thus an understanding of normal platelet function is a prerequisite to the development of the product, since to be effective a platelet substitute must be able to mimic the key processes in the formation of a haemostatic platelet plug. Furthermore a thrombus so formed must be capable of dissolution by normal fibrinolysis.

Platelets normally circulate in a resting state but, following vessel injury, they rapidly adhere to von Willebrand factor (vWF) on the damaged sub-endothelial cell surface, through the GPIbα platelet receptor. This

interaction occurs under conditions of high shear, such as is experienced in flow in damaged blood vessels, and its importance is demonstrated by the bleeding diathesis seen in patients with Bernard-Soulier syndrome (who lack the GPIb receptor) or severe von Willebrand's disease (who lack vWF). The process of this adhesion, together with the presence of a range of platelet agonists (collagen in the vessel wall, ADP released from damaged cells, thrombin generated locally by the interaction of exposed tissue factor with plasma clotting factors), causes activation of the platelets. This results in a conformational change in the GPIIb-IIIa receptor complex, allowing it to bind plasma fibrinogen and recruit further platelets into a growing thrombus. Platelets can also expose a negatively charged surface to which the prothrombinase complex can bind and generate thrombin, thus adding to the haemostatic plug by cleaving fibrinogen to form fibrin.

Platelet-platelet aggregation is critically dependent upon the interaction of fibrinogen with GPIIb-IIIa - the "final common pathway" of platelet activation. Coller (1980) *Blood* **55**, 2 demonstrated that inert beads coated with fibrinogen would bind to platelets through the GPIIb-IIIa receptor in the presence or absence of ADP, thus mimicking the action of platelets in causing aggregation.

Several attempts have been made to exploit this concept to develop a platelet substitute. RGD peptides (ie, peptides comprising the motif Arg-Gly-Asp), designed to interact only with the activated GPIIb-IIIa platelet receptor, have been covalently coupled to erythrocytes. Although the preparation could interact with activated platelets under conditions of low shear, it failed to reduce bleeding in thrombocytopenic primates. In a separate study, fibrinogen cross-linked to erythrocytes with formaldehyde augmented ADP- and thrombin-induced platelet aggregation, and shortened the bleeding time in thrombocytopenic rats. Whilst offering some promise,

both of these approaches have the disadvantage of still relying on cellular material.

WO 98/17319 discloses a product consisting of fibrinogen coated upon the surface of microcapsules of cross-linked human serum albumin. These were shown to interact with platelets under conditions of high shear, and significantly reduced the bleeding in thrombocytopenic rabbits. The fibrinogen-coated microcapsules were shown to interact with the GPIIb-IIIa receptor, because their binding to platelets was inhibited by an RGD-containing peptide. Partial blocking of activity by hirudin indicated that thrombin had cleaved the immobilised fibrinogen. The fibrinogen-coated microcapsules aggregated platelets in the presence of an agonist but were also able to induce platelet aggregation in the absence of agonists (i.e. were thrombogenic). This latter effect was variable and was batch and platelet donor dependent. Thus, although these studies indicated that these microcapsules could augment haemostatic plug formation, their interaction with non-activated platelets is an issue and could lead to adverse thrombotic events *in vivo*. It was clear from these data that further development of the product was required.

Additional analysis of the product of WO 98/17319 is described in Davies *et al* (*Platelets*, 2002, **13**, 197), in which the WO 98/17319 product is referred to as a "Synthocytes™". The ability of Synthocytes™ to induce platelet aggregation was analysed in inactive and activated platelets, i.e. in the absence or presence of the platelet activation agonist, ADP, respectively. Figure 2(A) of Davies *et al* reports the results of platelet aggregation assays in whole blood (WB), as measured by platelet counting techniques. Figure 2(A) shows that platelet aggregation for inactivate platelets (i.e. in the absence of ADP) is about 20% without Synthocytes™ and about 50% in the presence of Synthocytes™. In other words,

Synthocytes™ cause increased aggregation of inactive platelets. Under the specific test conditions, the increase in aggregation of inactive platelets was an approximate 2.5-fold. Figure 2(A) also shows the effects of Synthocytes™ on activated platelets. Platelet aggregation of activated platelets (i.e. in the presence of ADP) is about 40% in the absence of Synthocytes™ and about 70% in the presence of Synthocytes™. This is a less than 2-fold increase in platelet activation. Figure 2(A) of Davies *et al* demonstrates that Synthocytes™ have a platelet aggregating activity in the absence of ADP, i.e. they constitutively aggregate inactive platelets. Moreover, Figure 2(A) shows that Synthocytes™ cause a greater increase in the aggregation of inactive platelets (approximately 2.5-fold) than of activated platelets (less than 2-fold). These data demonstrate that Synthocytes™ do not bind (i.e. aggregate) activated platelets in preference to inactive platelets. Rather, the Synthoctyes™ of WO 98/17319 are constitutively active in the aggregation of platelets, irrespective of whether the platelets are active or inactive.

The object of the invention is thus to provide an improved platelet substitute. In particular, it is an object of the invention to addresses the need in the prior art for a safe non-thrombogenic platelet substitute.

Description of the Invention

The present invention provides an injectable pharmaceutical product comprising an agent, the agent comprising an insoluble carrier to which is bound a peptide, the peptide being capable of binding fibrinogen such that the agent binds to activated platelets in preference to inactivate platelets, and wherein the peptide is not fibrinogen.

In one embodiment, the peptide binds to the region of fibrinogen that is naturally bound either by the platelet membrane glycoproteins GPIIb-IIIa or by fibrin.

In a preferred embodiment, the peptide binds to the region of fibrinogen that is naturally bound by GPIIb-IIIa. The binding of GPIIb-IIIa to fibrinogen is discussed in Bennett, 2001, *Annals of NY Acad. Sci.*, **936**, 340-354.

The peptide may bind to one or both of the carboxy- or amino-terminal domains of the α-chain of fibrinogen. More particularly, the peptide may bind to an RGD-containing motif in one or both of said domains. The RGD-containing motif may have the sequence RGDX, where X is any amino acid, such as serine, valine, phenylalanine or alanine, and thus may be RGDF at amino acids 95-98, or RGDS at amino acids 572-575.

The peptide may bind to the C-terminal domain of the γ-chain of fibrinogen. More particularly the peptide may bind to a sequence within the final 15,12,10 or 4 amino acids of the C-terminal domain of the fibrinogen γ-chain. The final 12 amino acids are usually HHLGGAKQAGDV.

In another preferred embodiment, the peptide binds to the region of fibrinogen that is naturally bound by fibrin. The peptide may bind the D-domain of the γ-chain, such as between residues 337-379. The peptide may bind to the β-chain segment of the D-domain, such as the C-terminal region. The peptide may bind fibrinogen with a dissociation constant (K_d) of around 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 200, 250, 300, 350, 400 or more nM. A K_d of around 100 nM is preferred.

An agent of the invention binds fibrinogen such that, when bound, the fibrinogen binds activated platelets in preference to inactivate platelets.

Activated platelets are platelets in which changes resulting from stimulation by an agonist causes a change in the conformation of GPIIb-IIIa which then allows fibrinogen to bind and thus allows the platelets to aggregate , and in some cases release the contents of their intracellular granules, for example 5HT or to express granule membrane proteins on their surface, for example α-granule P selectin. The precise nature of the response varies between agonists and according to the doseof the agonist. Examples of agonists are thrombin, ADP and collagen. Platelets that are not activated have the potential to undergo such changes but have not yet been stimulated to do so by an agonist.

To test whether a peptide is capable of binding fibrinogen such that the fibrinogen has a binding preference for activated platelets, the test peptide is bound to a carrier according to the present invention and fibrinogen is allowed to bind the peptide, as described below, thereby to generate a test agent. The test agent is added to to platelets in suspension, for example in whole blood, platelet rich plasma or a suitable buffer solution in the presence or absence of an agonist of platelet activation, such as ADP (i.e. the test agent is added to activated or inactive platelets, respectively), and gently mixed, as described in Davies *et al*, 2002, *Platelets*, **13**, 197. The platelet suspension/test agent mixture is then analysed to determine whether the platelets are aggregated using the platelet counting technique described in Davies *et al.* As a control, the level of platelet aggregation is determined in the presence or absence of ADP, without adding the test agent, as described in Davies *et al.* The level of platelet aggregation correlates with the ability of the bound fibrinogen to bind a platelet. An agent according to the present invention, having a peptide that is capable of binding fibrinogen such that the fibrinogen has a binding preference for activated platelets, will show a bigger increase in aggregation between control and agent in the presence of ADP than in the absence of ADP. The skilled person will

appreciate that the same test can be performed using agonists of platelet activation other than ADP.

Typically, an agent according to the present invention, having a peptide that is capable of binding fibrinogen such that the fibrinogen has a binding preference for activated platelets, will cause less than a 150% increase in aggregation of inactive platelets, such as less than 140%, 130%, 120%, 110%, 100%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, 5%, 4%, 3%, 2%, 1% or substantially no increase in the aggregation of inactivated platelets compared to the inactive control level. Lower numbers are preferred. In this context, the basal level of aggregation of inactive platelets is taken to be 100%, and thus a 100% increase as defined above is a doubling (i.e. two-fold increase) in the level of aggregation and a 150% increase is a 2.5-fold increase in the level of aggregation.

An agent according to the present invention, having a peptide that is capable of binding fibrinogen such that the fibrinogen has a binding preference for activated platelets, may cause at least a 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% (i.e. 2-fold) increase, or more, in the aggregation of activated platelets compared to the activated control. Higher numbers are preferred. The level of increase in aggregation of activated platelets caused by an agent of the invention may be up to 50%, 60%, 70%, 80%, 90%, 100%, 150%, 200%, 250%, 300%, 350%, 400%, 450%, 500% or more.

In one embodiment, it is preferred that the agent of the invention is able to cause a greater fold-increase in the aggregation of activated platelets, compared to the activated control, than the inactive platelets, compared to the inactive control.

A product is an injectable pharmaceutical product if it is sterile, substantially pyrogen-free and has no medically unacceptable effects. For example, the product should not produce a medically unacceptable immunological reaction when injected into a human subject. Medically unacceptable effects can be determined by the skilled person in the field of medicine.

Importantly, in the case of the present invention, the fibrinogen-binding peptide of the agent should be capable of binding fibrinogen such that, if the agent has been loaded with fibrinogen via the fibrinogen-binding peptide and administered to a patient intravenously, the fibrinogen bound to the agent via the peptide should not be active in the formation of medically unacceptable levels of non-specific fibrin clots. By "non-specific", in this context, we include fibrin clot formation that occurs in the absence of active platelets at the site of a wound.

As discussed above, WO 98/17319 discloses fibrinogen-coated microcapsules, which induce platelet aggregation of inactive platelets (i.e. they are thrombogenic products). This is medically unacceptable. Without being bound by theory, we believe that the product of the present invention addresses the disadvantages faced by the prior art by binding fibrinogen in a conformation that does not result in medically unacceptable constitutive fibrinogen action. Accordingly, the fibrinogen-binding peptide bound directly to the carrier as used in the product of the invention is not fibrinogen, as defined in WO 98/17319.

In vivo, fibrinogen typically binds to platelets, which are activated by the presence of an agonist, such as ADP, thrombin, or collagen.

In one embodiment, if administered to a patient intravenously, the product will preferentially become involved in formation of a blood clot at the site of a wound where platelets are already activated. In this context, the phrase "preferentially becomes involved" means that, although low levels of binding of the product to inactive platelets may be acceptable, that level will not cause medically unacceptable levels of fibrin clot formation.

The fibrinogen-binding peptide as used in the product may comprise a sequence obtained from the platelet membrane glycoproteins GPIIb or GPIIIa (Bennett, 2001,*Annals of NY Acad. Sci.*, **936**, 340-354).

In particular, the fibrinogen-binding peptide may be obtained from fibrinogen-binding regions of GPIIb or GPIIIa. Preferred fibrinogen-binding regions include regions, which bind the α-chain amino, and/or carboxy-terminal domains of fibrinogen and regions that bind the γ-chain C-terminal domain of fibrinogen, as discussed above.

Thus the fibrinogen-binding peptide may comprise the sequence of AVTDVNGDRHDLLVGAPLYM, which represents the sequence of amino acids 294-314 of GPIIb, or a fibrinogen-binding fragment thereof. Such fragments include the sequence TDVNGDGRHDL (296-306), the sequence GDGRHDLLVGAPL (300-312) and the terminal tetrapeptide GALP. These sequences are thought to be involved in the binding of fibrinogen and, in particular, the γ-chain of fibrinogen (Bennett, 2001, *op. cit.*; D'Souzu *et al*, 1991, *Nature*, **350**, 66-68; Taylor & Gartner, 1992, *J. Biol. Chem.*, **267**, 11729-33). The similar effects of fragments 296-306 and 300-312 suggest that fragment 300-306 may also provide fibrinogen-binding activity.

The fibrinogen-binding peptide may comprise one or more of the peptides APLHK, EHIPA and GAPL which were shown in Gartner, 1991, *Biochem. Biophys. Res. Commun.*, **180**(3), 1446-52 to be hydropathically equivalent peptide mimics of the fibrinogen binding domain of GPIIb-IIIa.

The fibrinogen-binding peptide may comprise the sequence of residues 95-223 of GPIIIa or a fibrinogen-binding fragment thereof. For example, residues 211-222, comprising the sequence SVSRNRDAPEGG is thought to be an important fibrinogen-binding domain in GPIIIa (Charo *et al*, 1991, *J. Biol. Chem.*, **266**, 1415-1421).

Other suitable regions of GPIIIa include residues 109-171 and 164-202.

The skilled person will appreciate that fragments or variants of any of these sequences may also be used, so long as they provide fibrinogen-binding activity according to the present invention.

A particularly preferred fibrinogen-binding peptide comprises a sequence obtained from the platelet membrane glycoprotein GPIIb, namely TDVNGDGRHDL, or a variant of such a sequence.

Variants of TDVNGDGRHDL include –

T(D,E)VNG(D,E)GRH(D,E)L

TD(V, L)NGDGRHDL

TDV(N,Q)GDGRHDL

TDVNGDG(R,K)HDL

Such variants will have substantially the same fibrinogen binding activity as TDVNGDGRHDL, in that they will have substantially the same affinity for fibrinogen and, when bound, fibrinogen will have substantially the same

conformation and activity as when bound to TDVNGDGRHDL. By "substantially the same fibrinogen-binding activity" we include variants that bind fibrinogen with an affinity up to 1, 2, 3, 4, 5, 10, 50, 100 or more orders of magnitude different (either higher or lower) to TDVNGDGRHDL. Lower numbers are preferred.

Kuyas *et al*, 1990, *Thrombosis and Haemostasis*, **63**(3), 439, describes the use of the synthetic peptide GPRPK, immobilised via the C-terminal lysine to fractogel, to isolate fibrinogen from human plasma. Kuyas *et al* explains that human fibrinogen has a strong affinity for fibrin, and reports that the authors utilised a peptide comprising the N-terminal sequence of the α-chain of fibrin exposed by the action of thrombin, GPRP, which had been shown to bind fibrinogen (Laudano & Doolittle, 1980, *Biochemistry*, **19**, 1013; Laudano *et al*, 1983, *Ann. N.Y. Acad. Sci.*, **408**, 315). Kuyas *et al* concludes that the 'core' sequence GPR is required for fibrinogen binding.

Thus, the fibrinogen-binding peptide as used in the product may comprise the sequence of a fibrinogen-binding region of fibrin such as the N-terminal region of the α-chain or the C-terminal region of the β-chain. Accordingly the peptide may have the sequence Gly-(Pro/His)-Arg-Xaa at the amino terminus, wherein Xaa is any amino acid. In this context, by "at the amino terminus" we mean that the Gly residue in the above tetrapeptide sequence should represent the first amino acid of the peptide when read from the N-terminus to the C-terminus. By "Pro/His" we mean that either proline or histidine is included at that position. In one embodiment, proline is preferred to histidine.

Kuyas *et al* fails to disclose an injectable pharmaceutical product according to the present invention because the peptide is bound to Fractogel.

Fractogel is composed of polymethacrylate and has a minimum particle size of 20mm and would therefore not be pharmaceutically acceptable.

The peptide may comprise the sequence of Gly-Pro-Arg-Pro at the amino terminus.

Alternatively, the peptide may comprise the sequence of Gly-Pro-Arg-Sar (Sar is short for sarcosine, which is methyl glycine), Gly-Pro-Arg-Gly or Gly-Pro-Arg-Val at the amino terminus.

The peptide may comprise, in addition to a fibrinogen-binding sequence, an amino acid or sequence designed to aid attachment of the peptide to the carrier. For example, the peptide may include a terminal cysteine for linking to a thiol reactive group on the carrier (see below).

Typically the peptide has from 4 to 200 amino acids. Preferably, the peptide is no more than 150, 100, 90, 80, 70, 60, 50, 40, 30 or 20 amino acids in length. Preferably, the peptide is at least 4, 5, 6, 7, 8, 9, 10, 11 or more amino acids in length, although the minimum length should be at least long enough to include the fibrinogen-binding sequence in full.

The peptide may also comprise a spacer sequence. This can provide for spatial distances between the fibrinogen-binding sequence and the linkage to the carrier. This may aid in preserving the fibrinogen-binding activity of the peptide

The carrier should be insoluble, inert and biocompatible. The carrier should exhibit an insignificant effect on blood coagulation tested by adding the carrier to plasma and demonstrating no effect on the activated partial thromboplastin clotting time (APPT) using for example micronized kaolin

(supplied by Helena Laboratories Ltd.) to activate recalcified plasma or the prothrombin clotting time (PT) using for example Manchester thromboplastin reagent (supplied by Helena Laboratories). Similarly, the carrier should exhibit no effect on platelets when tested by the method of Davies *et al*, 2002, *Platelets*, 13, 197 as described above. The phrase "no effect" as used above includes the meaning that no the carrier has no medically unacceptable effect, as described above.

The carrier should have a size suitable to ensure transmission of the agent through the lung capillary bed. The ability of an agent to be transmitted through the lung capillary bed can be determined using the method of Perkins *et al*, 1997, *The British Journal of Radiology*, **70**, 603. Alternatively the ability of an agent to be transmitted through the lung capillary bed can be determined by injecting the agent into a host, for example an anaesthetised dog or cynamolgous monkey, and studying cardiovascular and respiratory safety, including an analysis of parameters such as blood pressure, pulse oximetry, respiratory and heart rate, and blood gas analysis. An agent that is able to be transmitted through the lung capillary bed, when injected into the host, will have substantially no effect on these parameters.

In this embodiment, the carrier may have a maximum dimension such that a minority, such as less than about 2% of the population by number, are in excess of 6 μm as a maximum dimension, as measured by particle counter, such as a Coulter Multizer II. A size of from 2 to 4 μm as a maximum dimension may be suitable, which is comparable to the size of human platelets.

In one embodiment, the carrier may be a microparticle. The term "microparticle" includes solid, hollow and porous microparticles. The

microparticles may be spherical (i.e. be "microspheres"), by which we include all substantially spherical shapes.

The microparticle may be formed of any suitable substance. It may be formed of cross-linked protein. A typical protein for these purposes is albumin, which may be serum-derived or recombinant and may be human or non-human in sequence. A protein microparticle may be formed by spray-drying protein. For example, microparticles suitable for use as a carrier by the present invention may be formed by spray drying human serum albumin, using well known spray-drying technology, such as in WO 92/18164. Accordingly, the carrier may be an albumin microparticle.

Alternatives to the use of microparticles as carriers include liposomes, synthetic polymer particles (such as polylactic acid, polyglycolic acid and poly(lactic/glycolic) acid), cell membrane fragments and the like.

The peptide can be bound to the carrier by any suitable means. The bond between the peptide and the carrier can be covalent or non-covalent. Typically the bond is covalent. A suitable covalent bond can be formed when the peptide comprises a cysteine and the carrier comprises a thiol reactive group. This allows the peptide to be bound to the carrier by linking the -SH group of the cysteine to the thiol reactive group on the carrier. Suitable thiol reactive groups include maleimide. An exemplary protocol for linking cysteines to thiol reactive groups is disclosed in Green *et al*, 1982, *Cell*, **28**, 477, although the skilled person will appreciate that any suitable method can be used.

A terminal cysteine residue may be incorporated in the fibrinogen-binding peptide to crosslink the peptide with thiol reactive groups on the carrier. Thiol reactive groups can be made available on the carrier using, for

example, maleimidobenzoly-N-hydroxsuccininmide ester (MBS) or succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate) (SMCC) crosslinkers to convert lysine residues in the carrier to thiol reactive maleimide groups. Alternatively the free thiol on, for example, an albumin carrier, can be used, a leaving group (for example 5,5'-dithio-*bis*(2-nitrobenzoic acid (DTNB) or Ellman's reagent) can be substituted into the free suphydryl group on the albumin carrier, and the cysteine in the peptide substituted for the leaving group.

An alternative method of linking the peptides to the carrier is to use a two step carbodiimide method (Grabarek, 1990, *Analytical Biochemistry*, **185**) in which the peptide is incubated with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N- hydroxysulphosuccinimide (sulpho-NHS), which results in the formation of an active ester. The peptide ester can be isolated and then mixed with the carrier and the ester allowed to react with amines on the carrier.

Any number of fibrinogen-binding peptides may be bound to the carrier. A platelet typically has 50,000-100,000 GPIIb-IIIa surface proteins. A similar number of fibrinogen-binding peptides on the carrier may be appropriate. For example, the carrier may have at least 1,000, 5,000, 10,000, 20,000, 30,000, 40,000, 50,000, 60,000, 70,000, 80,000, 90,000, 100,000 or more fibrinogenbinding peptides bound thereto, such as up to 50, 80, 90, 100, 110, 120, 130, 140, 150, 200, 400 or more thousand fibrinogen binding peptides. Around 80,000 may be preferred.

Fibrinogen, or a variant or fragment thereof, may be bound to the thus formed product, to provide an immobilised form of fibrinogen for administration to an individual.

This may be achieved by a method comprising the steps of providing a product as described above and mixing it with fibrinogen, or a variant of fragment thereof. In one embodiment, the fibrinogen (or variant or fragment) binds to the peptide as a result of the affinity of the peptide for the fibrinogen (or variant or fragment). Thus, the fibrinogen (or variant or fragment) may be bound to the peptide by non-covalent bonds. The non-covalent bonds can subsequently be stabilised by the formation of an additional covalent bond between the fibrinogen (or variant or fragment) and the peptide, or between the fibrinogen (or variant or fragment) and the carrier. Alternatively, the sole means of attachment of the fibrinogen (or variant or fragment) can be through a covalent bond to the peptide.

One suitable method for non-covalently attaching fibrinogen (or variant or fragment) includes incubating a product as defined above with blood, or plasma or a concentrate of plasma or recombinant fibrinogen, which is suitable for intravenous use at between 20°C and 37°C for an appropriate length of time. We have found that incubation for up to 3 hours at 20°C is satisfactory.

To achieve covalent attachment, fibrinogen can be crosslinked to the peptide, for example, using a zero length heterobifunctional crosslinker, such as EDC plus Sulpho-NHS as discussed above.

Subsequent steps in the production of an injectable pharmaceutical composition may include –

(a) removing unbound fibrinogen;

(b) formulating the product with a pharmaceutically acceptable carrier or diluent;

(c) diluting the product to provide a pharmaceutically acceptable unit dose; and

(d) sterilising the product.

It will be appreciated that steps (b) and (c) can be the same or different.

It will also be appreciate that step (d) may not be required. The product can be produced aseptically or alternatively terminally sterilised. An example of a suitable terminal sterilisation procedure is sterilisation as a liquid suspension by heating at a suitable temperature, for example 60°C for 10 hours or lyophilised first and heated to, for example, 80°C for 72 hours. Such procedures are commonly used to destroy viruses in blood proteins and would be expected to destroy bacteria. Alternatively gamma irradiation may be used, for example by exposure to 25-35Kgy using a cobolt^{60} source.

The form of attachment can be varied so long as the bound fibrinogen (or variant or fragment) binds to activated platelets in preference to inactivate platelets, and preferably, following intravenous administration, will only become involved in the formation of a blood clot at the site of a wound where platelets are already activated.

Accordingly, the product may additionally comprise fibrinogen, or a variant or fragment thereof, having an inducible platelet-aggregating activity, bound to the said peptide.

By "inducible platelet-aggregating activity", we mean that the fibrinogen binds to activated platelets in preference to inactivate platelets. Preferably, if administered to a patient intravenously, the fibrinogen portion of the

product will preferentially become involved in formation of a blood clot at the site of a wound where platelets are already activated. Methods for determining whether the platelet-aggregating activity of fibrinogen is "inducible" are discussed above.

The source of the fibrinogen can be, for example, a purified protein derived from plasma or blood or from a recombinant source. The fibrinogen may be human or non-human in sequence.

Any variant or fragment of fibrinogen may be used, provided that it has a useful level of inducible platelet-aggregating activity. In this context, a useful level of platelet-aggregating activity means that the variant or fragment can be used with the product of the invention to cause aggregation of activated platelets in preference to inactive platelets, as described above. Preferably, any such variant or fragment includes residues 398-411 of the gamma chain of fibrinogen. In a preferred embodiment, the variant or fragment may include, or even consist of, HHLGGAKQADV.

Accordingly, the present invention also provides an injectable pharmaceutical product having an inducible platelet-aggregating activity comprising an insoluble carrier to which fibrinogen, or a variant or fragment thereof, is bound in a configuration such that the fibrinogen (or variant or fragment) binds to activated platelets in preference to inactivate platelets. Typically the product, when introduced intravenously, will only become involved in formation of a blood clot at the site of a wound where platelets are already activated. The fibrinogen, or a variant or fragment thereof, is typically bound indirectly to the carrier through a fibrinogen-binding peptide as defined above.

In an alternative embodiment, a product as defined above may be administered without fibrinogen. In this case, the product is able to bind fibrinogen endogenous to the individual to whom the product is administered.

Accordingly, the present invention also provides a method of promoting haemostasis, i.e. improving the ability of an individual to produce fibrin clots, comprising administering a pharmaceutically effective dosage of a product as defined above. The product can thus be used to promote an individual's ability to form fibrin clots at wound sites, whilst avoiding medically unacceptable levels of non-specific formation of fibrin clots away from wound sites. Typically the method is a method of treating a patient with thrombocytopenia

Thrombocytopenia may result from conditions that cause increased platelet destruction. These include Immune thrombocytopenic purpura, disseminated intravascular coagulation, heparin-induced thrombocytopenia, other drug-induced thrombocytopenias, systemic lupus erythematosus, HIV-1-related thrombocytopenia, thrombotic thrombocytopenia purpura/haemolytic-uremic syndrome, common variable immunodeficiency, post-transfusional purpura, and type 2B von Willebrands disease.

Thrombocytopenia may result from conditions that cause decreased platelet production. These include thrombocytopenia with absent radii (TAR) syndrome, amegakaryocytic thrombocytopenia, giant platelet syndromes (such as Bernard-Soulier syndrome, May-Hegglin anomaly, Fechtner syndrome, Sebastian syndrome, Epstein syndrome, Montreal platelet syndrome), and Wiskott-Aldrich syndrome.

Thrombocytopenia may result from conditions that cause sequestration (for example, hypersplenism or Nasabach-Merritt syndrome) or increased platelet destruction and hemodilution (such as extracorporeal perfusion).

The method of the invention may also be used to treat a patient with any one of the above conditions.

However, the method may also be used to treat a patient with thrombasthenia (i.e. inherited or acquired). Acquired platelet function defects can result from uremia, myeloproliferative disorders (such as essential thrombocythemia, polycythemia vera, chronic myeloid leukaemia, and agnogenicmyeloid metaplasia), acute leukaemias and myelodysplatic syndromes, dysproteinemias, extracorporeal perfusion, acquired von Willebrands disease, acquired storage pool deficiency, antiplatelet antibodies, liver disease, drugs and other agents. Inherited platelet function defects can result from platelet adhesion conditions (such as Bernard-Soulier syndrome and von Willebrand disease), agonist receptor conditions (such as integrin $\alpha_2\beta_1$ (collagen receptor) deficiency, $P2Y_{12}$ (ADP receptor) deficiency or thromboxane A_2 receptor deficiency), signalling pathway conditions (such as $G_{\alpha q}$ deficiency, phospholipase C-β_2 deficiency, cyclooxygenase deficiency, thromboxane synthetase deficiency, lipoxygenase deficiency or defects in calcium mobilisation), secretion conditions (such as storage pool disease, Hermansky-Pudlak syndrome, Chediak-Higashi syndrome, Gray platelet syndrome, Quebec syndrome and Wiskott-Aldrich syndrome), aggregation conditions (such as Glanzmann thrombasthenia or congenital afibringenemia) and platelet-coagulant protein interaction conditions (such as Scott syndrome).

The method may also be used to treat a patient who has sustained mechanical damage to his/her platelets, such as occurs during extra corporeal circulation in coronary bypass surgery and/or haemodialysis.

Michelson, 2002, *Platelets*, Chapter 36: The Clinical Approach to Disorders of Platelet Number and Function, Elsevier Science (USA), 541-545, the contents of which are incorporated herein by reference, provides a review of various disorders of platelet number and function.

The present invention thus provides products as defined above for use in medicine. The present invention also provides products as defined above in the manufacture of a medicament for promoting haemostasis.

For example, present invention also provides products as defined above in the manufacture of a medicament for the treatment of a patient with thrombocytopenic condition, such as a condition described above.

Thrombocytopenia is diagnosed by counting blood cells. The normal platelet count is 150-400x10^9/l. Below this range primary haemostasis is impaired and bleeding time prolonged. However spontaneous life threatening bleeding will usually only occur when the platelet count drops under 10x 10^9/l.

Accordingly, a method or use as defined above can be applied when wherein the patient has a platelet count below 400x10^9/l, preferably below 150x10^9/l, and more preferably below 10x 10^9/l.

The most common cause of thrombocytopenia is a failure in platelet production from the bone marrow, such as in blood cancers or following cytotoxic chemotherapy or radiotherapy.

Accordingly, a method or use as defined above can be applied when the patient has a failure in platelet production from the bone marrow, such as is caused by a blood cancer, or cytotoxic chemotherapy or radiotherapy.

A method or use as defined above can be applied when the patient has an inherited or drug-induced disorders in platelet function, such as described above.

A method or use as defined above can be applied when the patient's platelets have been mechanically damaged, such as occurs during extra corporeal circulation in coronary bypass surgery or haemodialysis.

By "treat" as used above, we include the use of the above products in prophylaxis. Thus, for example, a product of the invention could be administered to a patient in advance of cytotoxic chemotherapy or radiotherapy, drug-induced disorders in platelet function, extra corporeal circulation in coronary bypass surgery or haemodialysis.

Characterisation of the composition of the agents of the invention

The fibrinogen content of the agents of the invention can be measured using a modified ELISA assay using an antibody suitable to bind a component in the carrier (for example, where the carrier comprises human albumin, then an anti-human HSA antibody may be used) as the solid-phase capture antibody, and HRP-conjugated rabbit anti-human fibrinogen antibody to detect the fibrinogen. Soluble fibrinogen bound to a plate can be used as a standard. Relative levels of bound fibrinogen can also be determined by flow cytometry, using FITC-conjugated rabbit anti-human fibrinogen (see below).

To determine whether the fibrinogen bound to the peptide on the agent of the invention can still be cleaved by thrombin to form fibrin, an agent of the invention can be treated with human thrombin. The material released will be analysed for fibrinopeptide A, using a commercially available ELISA method supplied by American Diagnostica. Cross-linking of the agent of the invention via fibrin-fibrin bridging can be tested in a modified agglutination assay, for example, a protocol based on Levi *et al*, 1999, *Nature Medicine*, **51**, 107-111.

The composition of the platelet substitute can be validated with respect to the pharmacological activity. That the immobilised fibrinogen exhibits the same characteristics as soluble fibrinogen with respect to the interaction with platelets can be demonstrated. Methods are described below which demonstrate that the product will interact with only with activated platelets (i.e. only in the presence of an agonist e.g. ADP or thrombin).

Assessment of activity of candidate preparations of the agent of the invention in specific in vitro assays:

A number of well-defined, *in vitro* assays can be used to evaluate different aspects of the interaction of the agent of the invention with platelets and the haemostatic system, namely platelet aggregation, platelet activation, platelet-dependent thrombus formation and adhesion under conditions of flow, thrombin generation and fibrinolysis.

Fresh platelets are used, obtained locally, from normal volunteers. Blood is collected by clean venepuncture, via a 21 gauge butterfly needle either into an anticoagulant (normally trisodium citrate, or anticoagulant citrate dextrose (ACD), and used within 15 minutes of collection. To study the

effect of the agent of the invention in blood with low platelet counts the platelets are depleted by centrifugation and the blood reconstituted in autologous plasma. Platelet counts are measured in all samples using an blood cell counter (AC^TDiff; Beckman-Coulter).

Assessment of agents of the invention on Platelet Aggregation

This method can be used to show that agent of the invention enhance platelet aggregation in the presence of an agonist only. A Beckman Coulter AC^TDiff and a PAP4 Bio/Data Corporation platelet aggregometer are available for measuring platelet aggregation in whole blood or in platelet-rich plasma (PRP) respectively.

The agent of the invention is added to either whole blood or platelet rich plasma and incubated at 37° C with stirring. Rate and % aggregation can be measured in PRP by measuring the increase in light transmission, in an aggregometer such as the PAP4. Alternatively if agent of the invention is added to whole blood, rate or % aggregation can be measured by counting residual platelets. Either spontaneous aggregation (in the absence of agonist) or aggregation in the presence of an agonist such as ADP (adenosine diphosphate), TRAP (thrombin receptor activation peptide) and CRP (collagen related peptide) can be determined.

Ideally, an agent of the invention, when tested by this method, will show a greater increase in the aggregation of active platelets than inactivated platelets as defined above.

Flow cytometric analysis of the interaction of the agent of the invention with platelets.

A Coulter Epics XL MCL Flow Cytometer can be used to examine of the stoichiometry of interactions between platelets and the agent of the invention, and to assay whether the agent of the invention causes a platelet activation, as demonstrated by a change in surface antigens e.g. P selectin in the absence of exogenous stimulation. P selectin is a marker of platelet degranulation and therefore platelet activation.

The agent of the invention is added to whole blood and mixed in a controlled manner, with and without exogenous agonists such as ADP, TRAP, and CRP. Aliqouts of these mixtures are diluted in HEPES buffered saline containing fluorescently conjugated Mabs and incubated to allow antibody binding. For example R-Phycoerythrin(RPE) conjugated mouse monoclonal to human GPIb (Cy5RPE) available from BD Biosciences, Oxford, UK and a FITC-labelled polyclonal antibody to human serum albumin (Autogen Bioclear Ltd) can be used to identify the platelets and agents of the invention respectively and to measure interactions between platelets and the agent of the invention with and without agonist stimulation. In addition FITC-conjugated Mabs to markers of platelet activation can be used to show whether platelets either free or bound to agent of the invention are activated. Activation of GPIIb-IIIa complex (a prerequisite of platelet aggregation) can be measured using a Mab PAC-1 which recognises an epitope on the GPIIb/IIIa complex of activated platelets at or near the platelet fibrinogen receptor (Becton Dickenson Immunocytometry Systems). Alternatively, a Mab specific for platelet P selectin can be used as a marker of platelet degranulation (Serotec).

Following incubation with the relevant antibody fluorescence is measured on the Flow Cytometer. Typically, agents of the invention will cause relatively little activatio of platelets as measured by PAC-1 Mab or P-selectin binding, as described above. In one embodiment, the term

"relatively little" means that agents of the invention cause less platelet activation than prior art platelet substitutes as defined above.

Preparation of a platelet-poor plasma sample

The agent of the invention is added to blood with abnormal levels of platelets, or to reconstituted blood which is artificially depleted in platelets. To artificially deplete platelets in normal blood, the blood is centrifuged at 100 × *g* for 20 minutes at room temperature. The platelet rich plasma is removed, and centrifuged at 7500 × *g* for 20 minutes and platelet poor plasma recovered in the supernatant. The platelet poor plasma is then added back to the red cell fraction and carefully mixed. A reduction of approximately 75% of the original platelet count is achieved by this method.

Study of the interaction of the agent of the invention with platelets under conditions of high shear rate flow:

A Parallel Plate Perfusion Chamber can be used to study the interaction of the agent of the invention with platelets, under conditions of flow, using variable rates of shear.

As a control, surface coverage is observed when a normal blood sample is perfused at different shear rates over sub-endothelial matrix or a collagen substrate (Levi et al, 1999, Nature Medicine, 51, 107-111). Then surface coverage is observed using the same protocol but with a platelet-poor sample prepared as described above. Surface coverage should be reduced in the platelet-poor sample, compared to the normal blood sample. Agents of the invention will preferably be able to increase surface coverage when added to the platelet-poor sample.

When the agents of the invention are added to the platelet-poor sample in sufficient numbers to stoichiometrically replace the depleted platelets (i.e. platelet-poor samples prepared as described above are reduced to a platelet count of about 25% of the undepleted sample: for the purposes of this test, agents of the invention are added in sufficient numbers to return the total number of remaining platelets plus agents of the invention to substantially 100% of the original platelet count in the undepleted sample), then it is particularly preferred if the agent of the invention is able to increase surface coverage in the platelet-poor sample to a level that is substantially equivalent to (e.g. 50, 60, 70, 80, 90 or 100%), or even higher than, the level of surface coverage observed for normal blood under the same flow conditions.

The extent of surface coverage by deposition of platelets or a combination of platelets and agents of the invention can be determined microscopically.

Evaluation of FLPs in vivo models:

Dose related haemostatic activity in a thrombocytopenic rabbit model:

Male New Zealand white rabbits 2.5-3.0kg (approximately 4 months old) are obtained from a reputable supplier. Groups of six rabbits are rendered thrombocytopenic using two doses of busulphan, 12 and 9 days respectively, prior to the study day. The dose of busulphan is varied according to the severity of thrombocytopenia required, e.g. two doses of 20mg/kg will generally reduce the platelet count to between 10-20 $x10^9/l$, whereas two doses of 25mg/kg will reduce the platelet count to less than $10x10^9/l$. In addition to a reduction in platelet count, busulphan dosing is associated with depletion of white cells, but only a minor reduction in

haematocrit and no overt toxicity. No anaesthetic is required for this procedure.

Human platelet concentrates are used as a positive control for these studies. This requires only one platelet concentrate per group of animals. It has been shown previously that human platelets circulate for only approximately 5 minutes in the rabbit, due to uptake by the reticulo-endothelial system. Therefore in these experiments macrophage function is inhibited by dosing the rabbits with ethyl palmitate 24 hours before the study day. For consistency, treatment with ethyl palmitate is used for all groups of animals.

On the study day the test agent is infused intravenously into an ear vein. Efficacy is assessed by measurement of bleeding time, which is performed using a standard (Simplate) incision in the ear.

Variability in the bleeding time is controlled, as far as possible, by ensuring that the animals are quiet and warm and at an even temperature (Roskam, 1993, *Comptes Rendus des Séances de la Societé de Biologie*, **114**, 166-169), and the number of blood samples minimised. Blajchman & Lee, 1997, *Transfusion Medical Reviews*, **11**, 99-105. Bleeding times are measured immediately prior to administration of the test dose and at four time points, up to 24 hours after dosing. Bleeding times in excess of 20 minutes are stopped by applying pressure to the wound. Animals are sacrificed at the completion of the study.

Dose related activity of the platelet substitute as defined by reduction in bleeding time is compared to the activity of human platelets (dose/kg basis). HSA microparticles of the same size but with no coupled peptide are used

as the negative control. A comparison of the duration of effect over the 24 hour period of the study is also made.

Assessment of the agent of the invention's thrombogenicity in a rabbit Wessler model

The potential thrombogenicity of the candidate FLP preparation is assessed in the Wessler model, essentially as described by Wessler *et al*, 1959, *Journal of Applied Physiology*, **14**, 943-946, but including controls appropriate to a platelet substitute. Controls are defined by consideration of data obtained from *in vitro* methods.

Male New Zealand White rabbits, body weight 2.5kg-3.0kg (approx 4 months) are obtained from an approved supplier and groups of six rabbits anaesthetised. Segments of the right and left jugular veins are exposed and detached from the surrounding tissue. The test preparations are administered through an ear vein and following a period of circulation of 3 minutes the segments of the jugular veins are ligatured and left *in situ* for a further 10 minutes. The segments are carefully excised, and the lumen exposed. The vessel is examined for the presence of developed thrombi, which is scored visually.

The minimum effect dose is identified, and a dose effect established in this model.

A suitable dose may be between 1×10^8 to 2×10^{10} product particles per kg of patient body weight. For example, the dose (when expressed a number of product particles per kg body weight) may be about 2×10^8, 3×10^8, 4×10^8,

5×10^8, 6×10^8, 7×10^8, 8×10^8, 9×10^8, 1×10^9, 2×10^9, 3×10^9, 4×10^9, 5×10^9, 6×10^9, 7×10^9, 8×10^9, 9×10^9, 1×10^{10} or 2×10^{10}.

An ideal dose has a safety margin of at least two-fold, preferably about 10-fold. In other words, the ideal dose is effective but remains safe even when increased by two-fold or about 10-fold. A safe dose does not form a clot using the Wessler test as described above.

CLAIMS

1. An injectable pharmaceutical product comprising an agent, the agent comprising an insoluble carrier to which is bound a peptide, the peptide being capable of binding fibrinogen such that the agent binds to activated platelets in preference to inactive platelets, and wherein the peptide is not fibrinogen.

2. A product according to Claim 1 in which, if the product is introduced intravenously, the peptide binds fibrinogen such that the bound fibrinogen will preferentially become involved in formation of a blood clot at the site of a wound where platelets are already activated.

3. A product according to Claim 1 or 2 wherein the peptide comprises a fibrinogen-binding sequence obtained from the platelet membrane glycoprotein GPIIb or GPIIIa, such as the sequence TDVNGDGRHDL or a variant of such a sequence.

4. A product according to any one of the preceding claims wherein the peptide comprises TDVNGDGRHDL.

5. A product according to any one of the preceding claims wherein the peptide comprises the sequence of Gly-(Pro/His)-Arg-Xaa at the amino terminus, wherein Xaa is any amino acid.

6. A product according to Claim 5 wherein Xaa is Pro, Sar, Gly or Val.

7. A product according to any one of the preceding claims wherein the peptide has from 4 to 200 amino acids.

8. A product according to any one of the preceding claims wherein the carrier has a size suitable to ensure transmission of the agent through the lung capillary bed.

9. A product according to any one of the preceding claims wherein the carrier is a microparticle.

10. A product according to Claim 11 wherein the microparticle is a protein microparticle, such as an albumin microparticle.

11. A product according to any one of Claims 8 to 10 wherein the wherein the product comprises a population of carriers of which less than 2% are in excess of 6 μm as a maximum dimension.

12. A product according to any one of Claims 8 to 11 wherein the carriers are from 2 to 4 μm as a maximum dimension.

13. A product according to any one of the preceding claims wherein the peptide is bound to the carrier by a covalent bond.

14. A product according to Claim 13 wherein the peptide comprises a cysteine and is bound to the carrier by linking the -SH group of the cysteine to a thiol reactive group on the carrier.

15. A product according to any one of the preceding claims wherein the product additionally comprises fibrinogen, or a variant or fragment thereof, having an inducible platelet-aggregating activity, bound to the said peptide.

16. A product according to Claim 15 wherein the fibrinogen (or variant or fragment) is bound to the peptide by non-covalent bonds.

17. A product according to Claim 15 or 16 wherein the fibrinogen (or variant or fragment) is bound to the peptide by covalent bonds.

18. An injectable pharmaceutical product having an inducible platelet-aggregating activity comprising an insoluble carrier to which fibrinogen, or a variant or fragment thereof, is bound in a configuration such that the fibrinogen binds to activated platelets in preference to inactive platelets.

19. A product according to Claim 18 which, when introduced intravenously, will only become involved in formation of a blood clot at the site of a wound where platelets are already activated.

20. A method for preparing a product as defined in any one of Claims 15 to 19, comprising providing a product according to any one of Claims 1 to 14 and mixing with fibrinogen, or a variant of fragment thereof and optionally further comprising one or more of the following steps –

(a) removing unbound fibrinogen;

(b) formulating the product with a pharmaceutically acceptable carrier or diluent;

(c) diluting the product to provide a pharmaceutically acceptable unit dose; and

(d) sterilising the product, or ensuring product sterility throughout steps (a) to (c).

21. A method of promoting haemostasis in an individual comprising administering to the individual a pharmaceutically effective dosage of a product as defined in any one of Claims 1 to 19.

22. A method of treating an individual with thrombocytopenia comprising administering a pharmaceutically effective dosage of a product as defined in any one of Claims 1 to 19.

23. A product as defined in any one of Claims 1 to 19 for use in medicine.

24. Use of a product as defined in any one of Claims 1 to 19 in the manufacture of a medicament for promoting haemostasis.

25. Use of a product as defined in any one of Claims 1 to 19 in the manufacture of a medicament for the treatment of a patient with thrombocytopenia.

26. A method or use according to any one of Claims 21 to 25 wherein the patient has a platelet count below $400x10^9/l$, preferably below $150x10^9/l$.

27. A method or use according to Claim 26 wherein the platelet count below $10x\ 10^9/l$.

28. A method or use according to any one of Claims 21 to 27 wherein the patient has a failure in platelet production from the bone marrow.

29. A method or use according to Claim 28 wherein the failure in platelet production from the bone marrow is caused by a blood cancer, or cytotoxic chemotherapy or radiotherapy.

30. A method or use according to any one of Claims 21, 23 or 24 wherein the patient has an inherited or drug-induced disorders in platelet function.

31. A method or use according to any one of Claims 21, 23 or 24 wherein the patient's platelets have been mechanically damaged.

ABSTRACT

THERAPEUTIC AGENT

The present invention provides an injectable pharmaceutical product comprising an agent, the agent comprising an insoluble carrier to which is bound a peptide, the peptide being capable of binding fibrinogen such that the agent binds to activated platelets in preference to inactivate platelets, and wherein the peptide is not fibrinogen.

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